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Mutations in the Coding Region of c-MYC in AIDS-associated and Other Aggressive Lymphomas

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Abstract

Our previous studies of the translocated MYC gene in Burkitt’s lymphoma showed the existence of clustered somatic mutations located in the transcriptional activation domain. We now report that aggressive lymphomas arising in the acquired immunodeficiency syndrome (AIDS) contain similar mutations and that the presence of mutations is correlated with the rearrangement of the oncogene. Mutations were also found in other de novo non-AIDS, non-Burkitt’s aggressive lymphomas with MYC rearrangements. An unusual asparagine to serine mutation at codon 11 was identified in several transformed follicular lymphomas without MYC rearrangement but not in normal tissues from patients with this mutation. These findings indicate that AIDS-associated and other de novo aggressive lymphomas with the MYC gene rearrangement are subject to the same mutation and selection process that affects Burkitt’s lymphomas.

Introduction

Recently, we and others have identified frequent, clustered mutations within the coding region of the translocated MYC gene in Burkitt’s lymphoma (1–3). These mutations are located within the 5’ and central portions of the second exon in regions critical to several functional activities of the MYC protein including cellular transformation (4), transcriptional activation (5), and apoptosis (6, 7). Functional studies from several laboratories indicate that selected mutations in these areas can result in an augmentation of the ability of MYC to mediate transformation (3, 9, 10). Although there is some controversy whether the basal transcriptional activation activity is affected (3, 8), it has recently been reported that mutations inhibit the ability of p107 to suppress MYC-induced transcriptional activation (11). These alterations of in vitro assays of MYC function suggest that the coding region mutations may play a role in its tumorigenic activity.

Other lymphomas besides classical Burkitt’s lymphoma possess rearranged MYC genes, but the existence of mutations in the MYC gene in these lymphomas is unknown. MYC gene rearrangements are particularly common in AIDS-NHL, occurring in 25–80% of cases (12, 13). These lymphomas have some molecular pathogenetic similarities to Burkitt’s lymphomas (13) but arise in a very different clinicopathological setting. Most of the cases with MYC rearrangement are classified as small, noncleaved cell lymphoma. However, other aggressive AIDS lymphomas, particularly diffuse large cell lymphoma of immunoblastic subtype may also show MYC rearrangement. We wished to determine whether the MYC gene in these AIDS-associated lymphomas was subject to mutation and, if so, whether the mutations were dependent on rearrangement of the oncogene. For this purpose, we examined 22 aggressive AIDS-associated lymphomas comprised mainly of small, noncleaved cell lymphoma and diffuse large cell lymphoma of immunoblastic subtype, with and without MYC gene rearrangement. To acquire additional information regarding the relationship of mutation to rearrangement, we also examined other types of non-AIDS-associated lymphomas with and without MYC gene rearrangement, including a group of de novo aggressive lymphomas and a group of progressed follicular lymphomas.

Materials and Methods

Cases Studied. Tissue samples from 51 patients at the NIH and the Los Angeles County-University of Southern California Medical Center were collected during standard diagnostic procedures. A total of 55 biopsies consisting of 22 AIDS-NHL (10 small noncleaved cell lymphomas, 11 large cell immunoblastic lymphomas, and 1 diffuse large cell lymphomas), 7 de novo diffuse aggressive lymphomas (3 large cell immunoblastic lymphomas, 1 small noncleaved non-Burkitt’s lymphoma, and 3 diffuse large cell lymphomas), and 22 aggressive TFLs were studied. Southern blot analysis of the restricted DNA was carried out to determine the involvement of EBV and the configuration of the immunoglobulin gene, MYC gene, and BCL-2 loci as described previously (14). All cases were monoclonal as assessed by immunoglobulin gene rearrangement studies. Nine of the 22 AIDS-associated lymphomas had a MYC rearrangement or cytogenetic evidence of 8q24 involvement. Five of the de novo aggressive lymphomas and four of the TFL had rearrangements of the MYC gene (15). All of the progressed follicular lymphomas, including those with MYC rearrangement, had involvement of the bcl-2 gene at the molecular or cytogenetic level. All patients in this study are or have been participants in clinical protocols and have been provided informed consent according to the Institutional Review Board of the NIH or the Los Angeles County-University of Southern California Medical Center.

PCR-SSCP Analysis of the Coding Regions of MYC. Oligonucleotide primers were synthesized by the solid-phase triester method. Nine sets of primers were synthesized that defined overlapping genomic segments designed to screen the entire MYC coding sequence (exons 2 and 3) using PCR-SSCP analysis. The sequences of the primers and the conditions for the SSCP analysis have been reported previously (1).

Subcloning and Sequencing of Mutations. All abnormal conformers identified in the genomic SSCP screening were sequenced following a subcloning procedure in which a PCR-generated genomic exon 2 or 3 fragment was cloned into the pAMP vector used in the CloneAmp system (GIBCO-BRL, Gaithersburg, MD; Ref. 16). This subcloning procedure has been described previously in detail (1). Prior to sequencing, 36 subclones were rescreened by SSCP to ensure that the correct conformer was present. To characterize each mutation, three or four subclones containing the same abnormal conformer that was present in the genomic DNA SSCP screening were sequenced using the dideoxy chain termination procedure and Sequenase 2.0 enzyme (United States Biochemical Corporation, Cleveland, OH). The subclone sequencing proved to be highly reproducible, as described previously (1). All abnormal conformers that reflected the pattern seen in the original tumor always generated the identical mutated sequence, whereas the normal wild-type conformers, sequenced for some cases, always showed wild-type sequence.

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2 The abbreviations used are: AIDS-NHL, acquired immunodeficiency syndrome-associated non-Hodgkin’s lymphoma; TFL, transformed follicular lymphoma; PCR-SSCP, polymerase chain reaction-single stranded conformational polymorphism.
Results

AIDS-NHL. Of the 22 AIDS-NHL studied, 9 had a MYC rearrangement or a cytogenetic translocation involving the 8q24 locus (Table 1). Abnormal SSCP conformers were identified in one or more of the second exon primer sets in 8 of the 22 AIDS-NHL (Fig. 1, Lanes 1–22). No abnormalities were detected in the SSCP analyzed using the third exon primers. Subsequent sequencing identified mutations in all eight cases. The majority of mutations (12 of 18) were observed in the 5' portion of the second exon between codons 56 and 129 (Table 2; Fig. 2). Three of the eight cases had missense mutations involving codon 56, 57, or 58; three others had missense mutations, and in one case, a deletion involved more central portions of exon 2, while two cases had a single sense mutation only. The distribution of mutation was similar to that which we and others described previously for the mutations identified in Burkitt’s lymphoma (1–3).

There was a strong predilection for mutations to be present in MYC rearranged cases. Six of 9 MYC rearranged cases displayed one or more mutations, while only 2 of the 13 MYC nonrearranged had mutations. The correlation of MYC rearrangement with mutation may be even stronger since, in the absence of cytogenetic studies for these cases, the possibility of a variant translocation involving MYC could not be eliminated. Mutations also preferentially involved the small noncleaved cell lymphomas, paralleling the higher percentage of MYC rearrangement in these lymphomas. However, even within this group, mutations occurred predominantly in MYC rearranged tumors, suggesting that the presence of mutation is more closely correlated with rearrangement than histology. Since most AIDS-NHL showed involvement by Epstein-Barr virus, there was no correlation of mutation with the presence of Epstein-Barr virus.

De Novo Aggressive Lymphomas, Non-AIDS. Of the seven patients with de novo aggressive lymphomas studied, five had rearrangement of MYC or translocations involving 8q24. Abnormal SSCP conformers were seen in two of the five MYC rearranged cases (Fig. 1, Lanes 23 (case D4) and 24 (case D5)). Again, all abnormalities occurred using the second exon primer sets. One case (Table 2, D4) was found to have three mutations and a previously described polymorphism at codon 33 (2) located within the 3’-region of the second exon, while the other case (Table 2, D5) had a single sense mutation located at codon 3. Thus, in this group, the MYC rearranged cases also appear to be subject to mutation. The number of cases studied, however, were too small to estimate the prevalence of mutation.

TFLs. Of the 22 patients with an aggressive B-cell lymphoma who had a previous histological diagnosis of follicular lymphoma, 4 had a MYC rearrangement (Table 1). None of these four cases showed any abnormality in the SSCP screening. To the contrary, four cases without MYC rearrangement showed abnormal SSCP conformers in

Table 1 Correlations between histology, MYC rearrangement status, Epstein-Barr virus (EBV), and the presence of MYC mutations in AIDS-NHL

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Histology*</th>
<th>MYC or 8q24</th>
<th>EBV status</th>
<th>MYC mutation</th>
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<tr>
<td>A1</td>
<td>SNC</td>
<td>+ (8;22)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A2</td>
<td>SNC</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A3</td>
<td>SNC</td>
<td>- (8;22)</td>
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<td>+</td>
</tr>
<tr>
<td>A4</td>
<td>SNC</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A5</td>
<td>SNC</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A6</td>
<td>SNC</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A7</td>
<td>IBL</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>A8</td>
<td>IBL</td>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A9</td>
<td>IBL</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>-</td>
</tr>
<tr>
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<td>SNC</td>
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<td>+</td>
<td>-</td>
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<td>+</td>
<td>-</td>
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<td>SNC</td>
<td>-</td>
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<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
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<td>+</td>
<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
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<tr>
<td>A18</td>
<td>IBL</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>A19</td>
<td>IBL</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>A20</td>
<td>IBL</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A21</td>
<td>IBL</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A22</td>
<td>IBL</td>
<td>+</td>
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</tbody>
</table>

* SNC, small noncleaved cell; IBL, diffuse large cell lymphoma of immunoblastic subtype; DL, diffuse large cell lymphoma.

Fig. 1. A, PCR-SSCP analysis of selected regions of the MYC second exon. a) shows the SSCP's generated using primers 2–1 and 2–2 as illustrated in the map below. b) shows the SSCP's using primers 2–3 and 2–4. Lanes 1–9, MYC rearranged AIDS-NHL corresponding to cases A1-A9 in Table 1; Lanes 10–22, AIDS-NHL without MYC rearrangement corresponding to cases A10-A22. Lanes 23 and 24, cases D4 and D5, the two non-AIDS de novo aggressive lymphomas found to have mutations. Lanes 25–30, six different biopsies from patients T20 (Lane 25), T21 (Lanes 26 and 27), and T15 (Lanes 28–30) showing identical abnormal conformers. Lanes 26 and 28, the preceding indolent follicular lymphomas phase from transformed cases T21 and T15, respectively, showing the presence of the same abnormal conformer seen in the transformed biopsies. Arrows, lanes with abnormal conformers. B, the full set of overlapping primers used for the second exon. The third exon primers are not illustrated. All primer locations and their sequences have been previously reported (1).
of nonlymphomatous normal tissues obtained at autopsy in two of the three cases (liver from case T15 and prostate from case T20) revealed only germline conformers in genomic SSCP analysis (data not shown). It is, therefore, likely that this codon 11 substitution represents a true mutation and not a polymorphism. The significance of this mutation, which occurred in 14% (3 of 22) of TFLs but was not observed in other lymphomas or controls reported in this or previous studies, is unknown. In a fourth case, a single missense mutation was found at codon 170 (Table 2, T13). Thus, none of the transformed cases showed mutations in the previously described cluster regions. The mutations present in the transformed follicular lymphoma group appear to be different from the mutations identified in the MYC rearranged lymphomas.

**Discussion**

Using SSCP analysis and sequencing, we have identified clustered mutations in the second exon of the MYC gene in a high percentage (36%) of aggressive lymphomas associated with AIDS. A total of 17 point mutations and 1 deletion was identified within the second exon in 8 of the 22 AIDS-NHL studied. Six of 9 tumors with MYC rearrangement had mutations, while only 2 of 13 nonrearranged cases were found to have mutations. None of the cases had mutations in the third exon. Mutations were primarily associated with tumors that possessed rearrangement of the MYC gene or a translocation involving 8q24. This report extends our previous finding describing similar mutations in a series of Burkitt’s lymphomas not associated with AIDS and suggests that the same processes that lead to mutations in Burkitt’s lymphoma also operate in MYC rearranged AIDS-related lymphomas.

The distribution of mutations in the AIDS-related group was similar to that reported previously in classical Burkitt’s lymphoma and mouse plasmacytomas (1–3). Mutations were most frequently observed in the 5’ region of the second exon, predominantly between codons 56–71 and 114–129. It is noteworthy that these regions of the MYC protein have been associated previously with several properties or functional activities of MYC, including cellular transformation (4), transcriptional activation (5), binding to the retinoblastoma gene product and the related protein p107 (17, 11), phosphorylation (18, 19), and apoptosis (20). Other investigators have shown that selected mutations in these regions generated by in vitro mutagenesis (3, 8–11) or derived from mutant MYC alleles (3, 11) can, in fact, affect some of these properties. Nonetheless, the contribution of the described mutations to in vivo pathogenesis remains to be determined.

Similar mutations were also found in a small group of MYC rearranged de novo aggressive lymphomas, but they did not appear to be present in a subset of four TFLs that had acquired MYC rearrangement at the time of progression. The latter finding is somewhat
surprising, given the relatively high rate of mutation that occurs in the immunoglobulin variable region gene segment of follicular lymphomas (21, 22). This may reflect differences in pathogenesis or simply be a statistical anomaly due to the small number of MYC rearranged tumors available in this group.

To the contrary, mutations were identified in four other cases of TFLs that did not have MYC rearrangement. Identical mutations were observed within codon 11 in six biopsies from three cases of TFL (T15, T20, and T21) which resulted in the substitution of a serine for the normal asparagine. In two cases, the same mutation was present in the patients’ preceding indolent follicular lymphoma biopsies. Since we could not identify this mutation in normal tissues from two of the three patients (data not shown), we believe that this substitution represents a true mutation and not a polymorphism.

This mutation appears peculiar to the TFL group. It is outside of the usual mutation cluster region found in the MYC rearranged AIDS-related and Burkitt’s lymphoma groups. Furthermore, we have not found this mutation in over 70 other lymphomas and normal tissues studied to date. Although its significance is unclear, the fact that the same mutation is present in 15% of the TFLs suggests that there is a strong selective pressure to retain mutations at this site. Further studies are necessary to determine whether these mutations affect any of the known functional activities of the MYC protein.

In conclusion, this study has revealed the presence of frequent mutations in the second exon of the MYC gene in the majority of MYC rearranged AIDS-NHL. This finding is similar to that observed previously for nonimmunodeficiency-related Burkitt’s lymphoma and may reflect a common pathogenetic development. In addition, we have found that these coding region mutations preferentially occur in MYC genes that have undergone prior rearrangement or translocation. This suggests that the mutations are not simply generated by random genetic instability in these high grade tumors but that their occurrence is greatly promoted by the proximity of the MYC gene to the immunoglobulin gene locus. Finally, we also identified an unusual codon 11 mutation in a subset of TFLs. This mutation is independent of MYC rearrangement and is not in the cluster region described previously for the MYC rearranged tumors, suggesting that its selection is occurring through a different mechanism.

References


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